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## *Abbreviations*

aa	Amino acid
ABCA1	ATP-binding cassette transporter 1
AChEI	Acetylcholinesterase inhibitor
AD	Alzheimer's disease
ADIT	Alzheimer's disease innovative drug target
AICD	APP intracellular domain
AMPARs	$\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AMPS	Ammonium persulfate
ANOVA	Analysis of variance
AP	Alkaline phosphatase
APH-1	Anterior pharynx-defective 1
APLP1	APP-like proteins 1
APLP2	APP-like proteins 2
ApoE	Apolipoprotein
APP	Amyloid protein precursor
ATP	Adenosine-5'-triphosphate
AVE	Anterior visceral endoderm
A $\beta$	beta-amyloid peptide
BACE-1	beta-site amyloid precursor protein cleaving enzyme 1
BBB	Blood-brain-barrier
Bp	Base pairs
BrdU	Thymidine analog bromodeoxyuridine
BSA	Bovine serum albumin
C°	Degrees Celsius
C1P	Ceramide-1-phosphate
CA1-2-3	Cornu Ammonis 1-2-3
CAA	Cerebral amyloid angiopathy
CamKII	Calmodulin-dependent protein kinase II
CamKII- $\alpha$	Calmodulin-dependent protein kinase II
cDNA	Complementary DNA
CLU	Clusterin
CNS	Central nervous system
COX	Cyclooxygenase
DAPI	4',6-diamidino-2-phenylindole
DDT	Dithiothreitol
DEPC	Diethyl pyrocarbonate
DG	Dentate gyrus
DKK1	Dickkopf- 1
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DS	Down Syndrome
DTT	Dithiothreitol
E	Embryonic day
EDTA	Diaminoethanetetra-acetic acid disodium salt
EGFP	Enhanced green fluorescent protein
EOAD	Early-onset Alzheimer's disease
EU	European Union
EU	European Union
FBS	Foetal bovine serum



FP6	Framework program 6
FTDP-17	Frontotemporal Dementia with Parkinsonism-17
G418	Geneticin selective antibiotic
GABA <sub>A</sub>	γ-aminobutyric acid
GFAP	Glial fibrillary acidic protein
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GNDF	Glial Cell derived Neurotrophic Factor
G-PCR	G-protein coupled receptor
GSAP	γ-secretase activating proteins
GSK3-α	Glycogen synthase kinase 3 alpha
GSK3-β	Glycogen synthase kinase 3 beta
GWAS	Genome-wide associated studies
hCMV	Human cytomegalovirus
HD	Huntington's disease
HTS	Hit-to Lead Phase
IHC	Immunohistochemistry
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-8	Interleukin 8
Kb	Kilo base
LB	Luria-Bertani
LIF	Leukaemia inhibitor factor
LOAD	Late-onset Alzheimer's disease
LTP	Long term potentiation
LTP	Long term potentiation
μg	Microgram
μL	Microlitre
μM	Micrometre
mES	Mouse Embryonic stem cells
MHCII	Major histocompatibility complex class II
mM	Millimolar
mM	Millimolar
mRNA	Messenger ribonucleic acid
MWM	Morris water maze
NF-κβ	Nuclear factor kappa beta
NFTs	Neurofibrillary tangles
ng	Nanogram
NGS	Normal goat serum
NMDARs	N-methyl D-aspartate
NSAID	Nonsteroidal anti-inflammatory drugs
p	Probability value
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PBST	PBS with Tween-20
PCR	Polymerase chain reaction
PD	Parkinson's disease
PFA	Paraformaldehyde
PGCs	Primordial germ cells
PHF	Paired helical filament
PICALM	Phosphatidylinositol binding clathrin assembly protein
PKA	Protein kinase A
PP-2A	Protein phosphatase 2
PS1	Presenilin 1

PS2	Presenilin 2
PVDF	Polyvinylidene fluoride
ROS	Reactive oxygen species
RT	Room temperature
RT-PCR	Reverse transcription-PCR
rt-TA	Tet on Transactivator
S1P	Sphingosine-1-phosphate
S1P3	Sphingosine 1-phosphate receptors 3
SB	Subiculum
SDS	Sodium dodecyl sulphate
SM	Sphingomyelin
SR	Serum replacement
TBST	Tris buffered saline with Tween-20
tg	Transgenic
Tg	Transgenic
TGFβ	Transforming growth factor beta
TM	Trans menbrane
Tm	Melting temperature
TNFα	Tumor necrosis factor-alpha
TRE	Tet systems response
tTA	Tet off Transactivator
U	Unit enzyme
UV	Ultraviolet
Volts	Volts
$\chi^2$	Chi-squared
$\tau$	Tau

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## *Abstract*

AD is thought to be caused by an abnormal production and aggregation of amyloid-beta (A $\beta$ ) peptide. Consequently, precluding the generation of A $\beta$  has been considered as a strategy for AD treatment. Pharmacological compounds reducing A $\beta$  formation have been developed and tested in preclinical and clinical trials and the often promising results obtained in preclinical trials have not been successful reproduced in clinical trials.

With this in mind in 2005 the European Framework Program 6 funded the ADIT project (Design of Small Molecule Therapeutics for the Treatment of Alzheimer Disease Based on the Discovery of Innovative Drug Targets) and this study has been part of this large collaborative study which involved eight institutions across Europe.

The main aim of the ADIT project was to identify new druggable targets for AD drug discovery. The project involved a screen for novel candidate AD target genes, performed by Siena Biotech and involved a number of other collaborating laboratories with roles in validating these targets. Validated targets were those genes: induced in response to A $\beta$  treatment of cultured neurons and with demonstrable neurotoxic activity; induced in the brains of AD patients and in brains from an existing mouse model of AD; deemed most tractable as targets of small molecule inhibitors. Two targets, DKK-1 and S1P3, fulfilled these requirements.

Drug discovery relies on animal models and the aim of this thesis was to develop transgenic mice for the selected targets and to investigate their role in AD pathology. Animal models were generating by pronuclear injection.

Preliminary findings suggest that S1P3 may contribute to the inflammatory process seen in AD. Chronic neuroinflammation is a common characteristic of AD and it may be responsible of the neuronal loss seen in AD. GFAP immunohistochemistry on brains of the S1P3 mice revealed a strong astrocytotic process particularly evident in the hippocampus (mainly in the dentate gyrus) Upregulation of GFAP is commonly accompanied by astrocyte proliferation and activation which leads to the production of pro-inflammatory and cytotoxic cytokines, as well as toxic molecules. A large body of evidence suggests that by transforming from a basal to a reactive state, astrocytes neglect their neurosupportive functions, thus rendering neurons vulnerable to neurotoxins, including proinflammatory cytokines and reactive oxygen species. The S1P3 mouse model represents a model for acquiring more insights into mechanisms of A $\beta$ -mediated toxicity in AD and a target for preventing astrocyte activation.

The characterization of the DKK-1 mouse model is still in its infancy, nevertheless preliminary analysis have already demonstrated that is up-regulation cause Glycogen synthase kinase-3 $\beta$

(GSK3- $\beta$ ) activation which in turn hyperphosphorylate tau. It is known that hyperphosphorylation of tau is responsible for NFTs formation and DKK-1 inhibition might prevent this process.